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CHARACTERIZATION OF PHOSPHATE SOLUBILIZING RHIZOBACTERIA AND ENDORHIZOBACTERIA FROM MEDICINAL PLANT (*PICRORHIZA KURROA*) AND THEIR EVALUATION AS ANTIFUNGAL AGENTS

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ABSTRACT

Phosphate solubilizing microorganisms play an important role in supplementing phosphorus to the plants by several mechanisms like lowering of pH by acid production and ion chelation and thus benefit plant growth and development. Therefore, the aim of this study was to determine the phosphate solubilizing potential of plant growth promoting rhizobacteria (PGPR) isolated from Picrorhiza kurroa and evaluate these isolates for their antifungal activity. Forty bacterial isolates were selected as the representative of the total plated population from the rhizosphere soil and rhizome/roots of the Picrorhiza kurroa from two locations of Chamba district. Four phosphate solubilizing bacterial isolates exhibited very good production of chitinase enzyme with a zone size of 30-45mm.Maximum IAA production of 30.00µg/ml was exhibited by PkR(34)* and PkR(7b)*. The isolate Pk12 (b) produced maximum per cent siderophore unit (27.21 %). All the bacterial isolates were able to grow on Pikovskaya's solid and liquid media and solubilized phosphorus but isolate PkR(7a)* showed maximum P- solubilization of 320.00 mg/l. Thee isolates viz., Pk14(a), Pk14(c) and PC7 had very high HCN production in which the colour of entire filter paper got changed from yellow to brown Out of the forty isolates, the isolates PkH(5), Pk12(d), Pk13(a), Pk13(b), PkR(33)*, PC4 and PC8 were found antagonistic against all the five fungal pathogens namely Alternaria solani, Fusarium oxysporum, Pythium aphanidermatum, Sclerotium rolfsii and Dematophora necatrix. Rests of the isolates were found with different degrees of antagonism against these five fungal pathogens.

KEYWORDS: Phosphate Solubilization, Rhizobacteria and Antifungal Activity

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Original Article

INTRODUCTION

The use of chemical fertilizers and fungicides might adversely affect the medicinal value of the plant. Using chemical fertilizers continuously in a particular soil, its nutrient value is reduced, posing a major threat to sustainable crop production. One of the most acceptable and environmentally conscious approaches in solving this problem is the use of naturally occurring microbial antagonists and plant growth promoting rhizobacteria. There is an urgent need to develop effective and more consistent active PGPR (plant growth promoting rhizobacteria) inoculants because there is an experimental support for the idea that PGPR may be used as bio-fertilizers or bio-control agents to increase survival and growth of medicinal plants under field conditions. Microbial inoculants or bio-fertilizers are used to hasten biological activity to improve availability of plant nutrient by fixing atmospheric nitrogen, making insoluble phosphates soluble and decomposing farm wastes which result in the release of plant nutrients.

Phosphorus is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al.,* 2002). Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition. Although microbial inoculants are in use for improving soil fertility during the last century, however, a meager work has been reported on P solubilization compared to nitrogen fixation. Soil P dynamics is characterized by physicochemical (sorption-desorption) and biological (immobilization-mineralization) processes. Large amount of Phosphorus applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils (Gyaneshwar *et al.,* 2002 and Hao *et al.,* 2002).

Efficiency of P fertilizer throughout the world is around 10 - 25 % (Isherword, 1998), and concentration of bioavailable P in soil is very low reaching the level of 1.0 mg kg^{-1} soil (Goldstein, 1994). Soil microorganisms play a key role in soil P dynamics and subsequent availability of phosphate to plants (Richardson, 2001). Phosphate solubilizing bacteria (PSB) are being used as biofertilizer since 1950s (Kudashev, 1956 and Krasilinikov, 1957). Release of P by PSB from insoluble and fixed / adsorbed forms is an important aspect regarding P availability in soils. There are strong evidences that soil bacteria are capable of transforming soil P to the forms available to plant. Microbial biomass assimilates soluble P, and prevents it from adsorption or fixation (Khan and Joergesen, 2009). Microorganisms enhance the P availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen *et al.,* 2006).Moreover, the problem of P fertilization may become serious in coming years because of the fact that manufacture of phosphate fertilizers require the use of non renewable resources such as high grade rock phosphate which are getting depleted progressively and are becoming costlier. In addition to this, chemical fertilizers have induced some environmental problems such as eutrophication of receiving water and number of health problems in living beings.

The solution for this major widespread problem is the use of biofertilizers because they are ecofriendly, non-hazardous and non-toxic to living beings. It has been reported that phosphate solubilizing microorganisms (PSM) play an important role in supplementing P to the plants allowing sustainable use of phosphate fertilizers (Kucey *et al.,* 1989). There are several studies indicating that soil inoculation with phosphate solubilizing bacteria improve solubilization of fixed soil P and result in higher yield of agricultural crops (Das *et al*., 2001; Bashan and Holguin, 2002 and Zayed *et al*., 2005).Though much information is available on activity of soil micro-organisms and plant growth promotion for annual crops (Glick, 1995 and Bashan, 1998), very limited information is available in respect of PGPR associated with medicinal plants such as *Picrorhiza kurroa.*

MATERIAL AND METHODS

Forty bacterial isolates obtained from rhizosphere of medicinal plant *Picrorhiza kurroa* and were evaluated for various plant growth promoting traits

Qualitative Estimation of Phosphate Solubilization (Plate Assay Method) (Pikovskaya, 1948)

The ability of bacteria to solubilize phosphorus was tested by streaking it on the PVK agar plates containing known amount of tricalcium phosphate (Ca_3 $(PO_4)_2$). The plates were incubated at 37^0C for 48h. Solubilization of phosphorus was observed by yellow coloured zones produced around the isolated bacterial colonies. Percent solubilization efficiency and phosphate solubilization index was calculated as:

$$SE\ (\%) = \frac{Z+C}{C} \times 100$$

SE = Solubilization efficiency

Z = Halozone diameter (mm)

C = Colony diameter (mm)

Tricalcium Phosphate Solubilization in Liquid Medium

Pikovskaya's medium was used for the solubilization of phosphate. Fifty ml of medium was dispensed in 250 ml of Erlenmeyer flask containing 0.5 per cent tri calcium phosphate (TCP) and autoclaved at 15 psi for 20 min. The flasks were inoculated with 10% per cent of the bacterial suspension and incubated at 35 ± 2^0C under shake conditions for 72 h. Simultaneously, one control of PVK broth was run, containing TCP but no inoculum. Flask contents were centrifuged at 15000 rpm for 20 min at 4^oC. The culture supernatant was used for determination of the soluble phosphorus as described by Bray and Kartz (1945). Estimation of soluble phosphorus formed by the action of phosphate solubilizing bacteria on tri-calcium phosphate was done calorimetrically and the results were extrapolated by standard curve drawn using potassium di-hydrogen phosphate.

P solubilizaton = T- C

T = PVK with TCP, inoculated

C = PVK with TCP, un-inoculated

Estimation of Siderophore by Chrome-Azurol-S (CAS) plate assay (Schwyn and Neilands, 1987)

Siderophore production was detected by CAS plate assay method. Sterilized blue agar was prepared by mixing CAS (60.5 mg/50ml distilled water) with 5ml iron solution (1mM $FeCl_3.6H_2O$) and 5ml 10mM HCl). This solution was slowly added to hexadecyltrimethyl ammonium bromide (HDTMA) (72.9 mg/40ml distilled water). Thus, 50 ml CAS dye was prepared and poured into 500 ml nutrient agar and the plates were prepared. Twenty-four hours old culture of the test bacteria was spotted on pre-poured blue coloured CAS agar plates. Plates were incubated for 72 h, at 37^0C. Formation of a bright zone with a yellowish, pinkish and whitish colour in the dark blue medium indicated the production of siderophore.

Quantitative Estimation of Siderophore Using Chrome-Azurol-S (CAS) Liquid Assay Method (Schwyn And Neilands, 1987)

0.1 ml of cell free extract of supernatant was mixed with 0.5 ml Chrome-azurol-S (CAS) assay solution along with 10 µl of shuttle solution (0.2M 5-Sulfosalicyclic acid). It was kept at room temperature for ten minutes and absorbance was recorded at 630 nm The minimal medium was used as a blank and the reference (r) was prepared using exactly the same components except the cell free extract of culture supernatant. The siderophore units were calculated using formula:

$$\text{Percent Siderophore unit} = \frac{Ar-As}{As} \text{ X } 100$$

A_r is defined as absorbance at 630nm of reference.

A_s is the absorbance at 630nm of the test.

Quantitative Estimation of Indole-3-Acetic Acid (IAA) (Gorden and Paleg, 1957)

For the production of auxins, bacterial cultures were grown in Luria Bertani broth (amended with 5 mM L-tryptophan, 0.065 per cent sodium dodecyl sulphate and 1% glycerol) for 72 h at 37^0C under shake conditions. Supernatant was prepared/ collected by centrifugation of cultures at 15,000 rpm for 20 minutes and was stored at 4^0C.In measuring the IAA equivalents, 3 ml of supernatant was pipetted into test tube and 2 ml of Salkowski's reagent (2 ml of 0.5 M $FeCl_3$ + 98 ml 35% $HClO_4$) was added to it. The tubes containing the mixture left for 30 minutes (in dark) for the development of pink colour. Intensity of the colour was measured at 535 nm. Concentration of Indole-3-acetic acid was estimated by preparing calibration curve using Indole-3-acetic Acid (IAA, Hi-media) as standard (10-100µg/ml).

HCN Production (Baker and Schippers, 1987)

The bacterial cultures were streaked on prepoured plates of King's medium B amended with 4.4g/L glycine. Whatman No.1 filter paper strips were soaked in 0.5% picric acid in 0.2% sodium carbonate and was placed in between the petriplates. Petriplates were sealed with parafilm and were incubated at 37^0C for 1-4 days. Un-inoculated control was kept for comparison. Plates were observed for change of colour of filter paper from yellow to orange brown to dark brown.

Chitinase Assay (Robert and Selitrennikoff 1988)

The bacterial cultures were spotted on to the prepared minimal agar medium amended with 0.3% colloidal chitin and the plates were incubated at 30^0C for 7 days. Development of halo zone around the colony after addition of iodine was considered as positive for chitinase enzyme production.

Bacterial Isolates as Antagonists against Various Pathogens

Agar Streak Method

In order to test the efficacy of the rhizobacterial antagonists, a loopfull of 48h old culture of each isolate was streaked a little below the centre of the prepoured petriplates (MEA) and then kept for overnight incubation at 37^0C to check for contamination. Mycelial disc of 4 days old culture of the test fungal pathogen was placed simultaneously on one side of the streak. A check inoculated with the test pathogen only was kept for comparison. Each treatment was replicated 3 times. The plates were incubated at 24 ± 1^0C and per cent growth inhibition was calculated according to Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

I= Per cent growth inhibition

C= Growth of fungus in control

T= Growth of fungus in treatment

RESULTS AND DISCUSSIONS

Forty isolates were eventually selected from the master plates (nutrient agar). The morphologies of selected bacterial isolates (Table 1) associated with *Picrorhiza kurroa* collected from two different locations were identified. The majority of twenty eight isolates (70%) were from rhizosphere soil and twelve (30%) isolates were from endorhizosphere (isolated from internal root tissues). All the representative isolates were gram positive rods and variable colony morphology. These forty phosphate solubilizing bacterial isolates were selected for further screening.

Phosphate Solubilization (Quatitative and Qualitative Both)

The phosphate solubilizing activity of the selected bacterial isolates were compared on the basis of their phosphate solubilizing index (PSI) in PVK agar Medium and P- solubilization (mg/l) in PVK broth medium. The results (Table 2) revealed that in PVK agar medium, Pk14(b) showed maximum phosphate solubilizing index (4.05) and minimum was recorded in Pk4a (2.27).The PSI of isolates Pk11(c) (3.0), Pk13(a) (3.16), Pk14(a) (3.0), Pk14(c) (3.42), PkR(7c)*(3.3), PC2 (3.0), PC7 (3.07), PC9 (3.05) and PC13 (3.42) was found statistically at par with PSI of isolate Pk14(b) (4.05).

In liquid PVK medium, maximum P- solubilization was recorded for endophytic isolate PkR(7a)* (320.0 mg/l) followed by Pk14(b) (205.0 mg/l) which was significantly lower than PkR(7a)*. The isolate Pk14(c) solubilized minimum TCP with release of 40.0 mg/l phosphorus.TCP solubilization of all the isolates was significantly lower than TCP solubilization in PkR(7a)*(320.0 mg/l).The maximum PSI was not related to the maximum P solubilization in liquid medium. The correlation coefficient (r=0.09) between PSI on solid medium and P solubilization in liquid medium by the bacterial isolates was found to be positive and non significant.

Siderophore Production (Quatitative and Qualitative Both)

The siderophore production of the selected bacterial isolates were compared on the basis of their zone size (mm) and per cent siderophore unit. All the isolates produced siderophore. The results (Table 2) revealed that endophyte PkR(33)* produced maximum zone size (24.0mm) which was statistically at par with 21.0 mm for PC7 and PC9 on Chome-azurol-S (CAS) solid medium and minimum zone size was observed in isolate Pk8(e) (3mm) .Quantitative estimation of siderophore using Chome-azurol-S (CAS) liquid assay revealed that Pk12 (b) produced maximum per cent siderophore unit (27.21 %) which was statistically at par with 20.0 per cent siderophore unit for isolate PK12(c). Minimum per cent siderophore unit (0.75 %) was found for isolates PkR (7a)* and Pk8 (e). The correlation coefficient (r=0.10) between qualitative and quantitative siderophore estimation was found to be positive and non significant.

Indole Acetic Acid Production (Quatitative)

The bacterial isolates showed large variation in their production of indole-3-acetic acid (IAA).The isolates PkR(34)* and PkR(7b)* produced 30µg/ml of IAA ,while in isolates PkR(32)* and PC2 no IAA production was observed. The isolate PC9 produced 28 µg/ml of IAA and was statistically at par with the amount of IAA produced by isolates PkR(34)* and PkR(7b)*. The majority of the selected P-solubilizing isolates (95%) produced IAA to various extents.

The chitinase activity of all the forty isolates (Table 3) which is expressed as zone of clearance (mm) was found of varying ranges. The chitinase activity was found in 11 isolates (91.66%) out of selected 12 endophytes and only in 21 rhizosphere soil (75%) isolates out of total 28 isolates selected. The highest chitinase activity was observed in case of isolates Pk4a, PkR(6a)*,PkR(7a)*and PkR(7b)* with a zone size ranging between (30-45mm).The isolates Pk3A, PkH(4), PkH(5), Pk6(B), Pk7(B),Pk11(c), Pk12(a), Pk12(c), Pk12(d), PkR(22)*, PkR(32)*, PkR(34)*, PkR(5a)*, PkR(5e)* , PkR(7c), PC2, PC7 and PC8 had zone size ranging between 15-30mm and the isolates Pk6(c), Pk8(a), Pk12(b), Pk13(a), Pk13(b), Pk13(c),Pk14(c), PkR(2)*, PkR(33)* and PC13 were having zone size ranging between 0-15mm.However,the isolates Pk8(e), Pk9(B), Pk14(a), Pk14(b), PkR(21)*, PC3, PC4 and PC9 had no chitinase activity. The isolates also showed great variation for HCN production (Table 3). Thee isolates (7.5%) viz., Pk14 (a), Pk14(c) and PC7 had very high HCN production in which the colour of entire filter paper got changed from yellow to brown. Seven isolates (17.5%)

(Pk3A, Pk11(c), Pk12 (a), Pk14 (b), PkR (2)*, PC4 and PC8) showed change in colour of half of the filter paper from yellow to brown. Only the edges of the filter paper turned brown in case of 17 isolates (42.5%)(Pk4a, PkH(4), PkH(5), Pk7(B), Pk9(B), Pk12(b), Pk12(c), Pk12(d), Pk13(a), PkR(21)*, PkR(33)*, PkR(34)*, PkR(5e)*, PkR(6a)*, PkR(7b)*, PkR(7c) and PC2). However,13(32.5%) isolates Pk13(b), PkR(5a)* ,Pk13(c), PkR(7a)*, PC3, PC9, PC13, Pk6(B), Pk8(A), Pk6(c), Pk8(e), PkR(32)* and PkR(22)*did not show HCN production activity.Overall,27 isolates (67.5%) had the ability to produce HCN out of which 8 isolates (20%) were endophytes.

All the forty isolates were screened for their antifungal activities against five fungal pathogens viz.,., *Fusarium oxysporum*, *Alternaria solani*, *Pythium aphanidermatum*, *Sclerotium rolfsii and Dematophora necatrix* (Plates 1, 2, 3 and 4).Table 4 revealed that out of all the forty isolates, 7 (17.5%) isolates PkH(5), Pk12(d), Pk13(a), Pk13(b), PkR (33)*, PC4 and PC8 were found antagonistic against all the five fungal pathogens. Of the seven bacterial antagonists, only one was endophytic. The isolates Pk6(c), Pk8(A), Pk8(e), Pk12(c), Pk14(b), PkR(32)*, PkR(5a)*, PkR(6a)*, PkR(7a)*, PkR(7b)* and PkR(7c)* were found to have no antifungal activity. Rest of the isolates were having varying degrees of antifungal activities. The isolates PkH(4), Pk11(c), PkR(34)*, PC3, PC4 and PC8 were having highest growth inhibition of fungus *Alternaria solani* accounting for 65-85 percent growth inhibition. Similarly, the isolates Pk3A, Pk14(c), PkR(21)*, PkR(34)* and PC4 had highest antifungal activity against *Sclerotium rolfsii.* However, the fungus *Dematophora necatrix* was greatly inhibited by 13 (32.5%) isolates i.e Pk4a, PkH(4), PkH(5), Pk6(B), Pk7(B), Pk11(c), Pk12(d), Pk13(a), Pk13(b), PkR(22)*, PkR (33)*, PC4 and PC7 upto the degree of 65-85 percent. Of these 13 bacterial antagonists only 2 (15.3) isolates were endophytic.

All the 40 representative phosphate solubilizing bacterial isolates were Gram positive rods and had variable colony morphology (Table 1).The most predominant P-solubilizing rhizobacterial isolates from *Picrorhiza kurroa* growing in wild habitat in location under this study were Gram positive rods. The dominance of genus *Bacillus* as a P solubilizing bacteria in the rhizosphere of several crops has been reported earlier (Illmer and Schinner, 1992; Motsara *et al*., 1995 and Tilak and Reddy, 2006). The majority of P solubilizing bacteria associated with *Salix alba* from Lahaul and Spiti valleys of Himachal Pradesh were reported to be Gram positive rods (Chatli *et al*., 2008).

Screening of the most efficient PSB *in vitro* was based on the ability of the isolate to release phosphorus into the culture medium and its relationship with the phosphate solubilizing index (PSI) based on colony diameter and halozone for each isolate. Since, in some cases, there have been contradictory results between plate halozone detection and P-solubilization in the liquid culture (Rodriguez and Fraga, 1999).

In the present study, positive but non-significant correlation (r=0.09) was observed between qualitative and quantitative P- solubilization. This finding is in support to the moderate positive correlation between the TCP solubilizing efficiency on solid medium and amount of phosphate solubilized in liquid medium by pseudomonads (Dave and Patel, 1999) and least correlation was found with P- solubilized and colony + halozone diameter (Alam *et al*., 2002 and Srivastav *et al*., 2004).

Our finding is in contrast to the pattern of phosphate solubilization by PSB in qualitative assay correlated well with the quantitative assay (Edi- Premono *et al*., 1996; Kumar and Narula, 1999 and Mehta and Nautiyal, 2001). This is because most PSB show fluctuation in their behavior of production of halozone on solid medium but it is not necessary that PSB with larger halozone would solubilize more phosphorus. In the present study, a positive non significant correlation (r=0.10) was observed between qualitative and quantitative siderophore estimation.

The exhibition of multiple plant growth promoting traits by a single strain of PGPR has been reported earlier also (Hamdali *et al*., 2008). Bacterial plant growth promotion is a well established and complex phenomenon, and is often achieved by the activities of more than one plant growth promoting traits exhibited by associated bacterium (Dastager *et al*., 2011)

Vassilev *et al*. 2006 for the first time demonstrated the capacity of *B.thuringiensis* to solubilize insoluble inorganic phosphate and simultaneously produce IAA in a repeated batch fermentation process. n The capacity to produce phytoharmones like IAA, is a desirable characteristic of PGPR (Vessey, 2003).

Almost all the selected bacterial isolates also showed antifungal activity against *Fusarium oxysporum*, *Pythium aphanidermatum*, *Sclerotium rolfsii*, *Alternaria solani* and *Dematophora necatrix* (Table 4). This is in agreement with those of (Virdi *et al*., 1994; Quesado *et al*., 1996; Khan and Khan, 2002; Ramirej *et al*., 2004 and Cazorla *et al*., 2007) who reported that *Bcaillus* strain UCR 236 and *Bacillus* spp. produced antifungal substances with activity against a number of mycelial fungi. A relatively wide range of antagonistic performances among the isolates has been observed in the present study and has also been noted in other studies involving the same or different fungi (Idris *et al*., 2007 and Calvo *et al*., 2010)

CONCLUSIONS

Out of forty phosphate solubilizing bacterial isolates, three isolates (Pk7(B), Pk14(b), and PC4) possess the capability to perform main plant growth promoting functions viz. solubilization of P, IAA production, siderophore production and antifungal activity against all the five fungal pathogens viz., (*Fusarium oxysporum*, *Alternaria solani*, *Pythium aphanidermatum*, *Sclerotium rolfsii* and *Dematophora necatrix*).The isolate PkR(7a)*, possessed very high ability to solubilize phosphate (320 mg/l) and average antifungal antibiotic production but lacked siderophore production. The isolate Pk14 (b) produced small amount of siderophore but lacked antifungal activity.

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REFERENCES

1. *Ezawa T, Smith S E and Smith F A. 2002. P metabolism and transport in AM fungi. Plant and Soil* ***244****:221-230.*
2. *Gyaneshwar P, Kumar G N, Parekh L J and Poole P S. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant and Soil* ***245****:83-93.*
3. *Hao X, Cho C M, Racz G J and Chang C. 2002. Chemical retardation of phosphate diffusion in an acid soil as affected by liming. Nutr. Cycl. Agroecosys.* ***64:****213-224.*
4. *Isherword K F. 1998. Fertilizer use and environment.* ***In****: N Ahmed and A Hamid (eds.), Proc.Symp. Plant Nutrition Management for Sustainable Agricultural Growth. NFDC, Islamabad. pp. 57-76.*
5. *Goldstein A H. 1994. Involvement of the quinoprotein glucose dehydrogenises in the solubilization of exogenous phosphates by gram-negative bacteria.* ***In****: A Torriani Gorini, E Yagil and S Silver (eds.), Phosphate in Microorganisms: Cellular and Molecular Biology. ASM Press, Washington, D. C. pp. 197-203*
6. *Richardson A E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol.* ***28****:897-906.*

7. *Kudashev I S. 1956. The effect of phosphobacterin on the yield and protein content in grains of Autumm wheat, maize and soybean. Doki. Akad. Skh. Nauk.* ***8****:20-23.*
8. *Krasilinikov N A. 1957. On the role of soil micro-organisms in plant nutrition. Microbiologiya* ***26****:659-72.*
9. *Khan K S and Joergensen R G. 2009. Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. Bioresource Technology* ***100****:303-309.*
10. *Chen Y P, Rekha P D , Arunshen A B, Lai W A and Young C C.2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol.* ***34****:33-41.*
11. *Illmer P and Schinner F. 1992. Solubilization of inorganic phosphate by microorganisms isolated from forest soils. Soil Biol. Biochemistry* ***24****: 389-395.*
12. *Motsara M R ,Bhattacharyya P B and Srivastava B. 1995. Biofertilizers- Their description and characterstics. In: Biofertilizer technology, Marketing and usage, A sourcebook-cum-glossary. Fertilizer development and consultation organization 204-204A, Bhanot Corner, 1-2 Pamposh Enclave, New Delhi, 110048, India, pp: 9-18.*
13. *Tilak K V B R and Reddy B S. 2006. Bacillus cereus and Bacillus circulans – novel inoculants for crops. Current Science* ***90****(5): 642-644.*
14. *Chatli A S, Beri V and Sidhu B S.2008.Isolation and characterisation of phosphate solubilising microorganisms from the cold desert habitat of Salix alba Linn.in trans Himalayan region of Himachal pradesh.Indian J.Microbiol.****48****:267-273.*
15. *Rodriguez H and Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances* ***17****(4-5): 319-339.*
16. *Dave A and Patel H H. 1999. Inorganic phosphate solubilizing Pseudomonads. Indian Journal of Microbiology* ***39****: 161-164.*
17. *Alam S, Khalil S, Ayub N and Rashid M. 2002. In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. International Journal of Agriculture and Biology* ***4****(4): 454-458.*
18. *Srivastav S, Yadav S and Kundi B S. 2004. Prospects of using phosphate solubilizing Pseudomonas and biofungicide. Indian Journal of Microbiology* ***44****: 91-94.*
19. *Edi-Premono M A, Moawad and Vleck P L G. 1996. Effect of phosphate solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. Indonasian Journal of Crop Science* ***11****: 13-23.*
20. *Kumar V and Narula N. 1999. Solubilization of inorganic phosphates and growth emergence of wheat as affected by Azotobacter chroococcum mutants. Biology Fertility of Soils* ***2.8****: 301-305.*
21. *Mehta S and Nautiyal C S. 2001. An efficient method for qualitative screening of phosphate solubilizing bacteria. Current Microbiology* ***43****: 51-56.*
22. *Vessey J K.2003.Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil* ***255****(2):571-580.*
23. *Vassilev N, Nikolaeva I, Vassileva M.2006.Phosphate solubilization and simultaneous indole-3-acetic acid production by a gel-entrapped bacterium in fermentation conditions. Chemical Engineering Communication(in press).*
24. *Kucey M N, Janzen H H and Legett M E. 1989. Microbially mediated increase in plant available phosphorus. Advanced Agronomy* ***42****: 198-228.*
25. *Das A K, Bera M K and Mohinddin M. 2001. Effect of different yield attributes on the productivity of wheat as influenced by growth regulators and biofertilizers. Environmental Ecology* ***19*** *: 145-148.*
26. *Bashan Y and Holguin G. 2002. Plant growth promoting bacteria: a potential tool for arid mangrove reforestation. Trees* ***16*** *:*

159-166.

27. *Zayed G and Motaal H A. 2005. Bioactive composts from rice straw enriched with rock phosphate and their effect on the phosphorus nutrition and microbial community in rhizosphere of cowpea. Bioresource Technology **96**: 929-935.*
28. *Glick B R, Jacobson C B, Schwarze M M K and Pasternak J J. 1995. 1- Aminocyclopropane-1-carboxylate deaminase mutants of the plant growth promoting rhizobacteria Pseudomonas putida GR 12-2 do not stimulate Canola root elongation. Canadian Journal of Microbiology **40**: 911-915.*
29. *Bashan Y. 1998. Inoculants of plant growth promoting bacteria for use in agriculture. Biotecnol. Adv. **16**: 729-770.*
30. *Hamdali H, Hafidi M, Virolle M J and Ouhdouch Y. 2008. Rock phosphate-solubilizing Actinomycetes: screening for plant growth-promoting activities. World Journal of Microbiology and Biotechnology **24**(11): 2565-2575.*
31. *Dastager S G, Deepa C K and Pandey A.2011.Potential plant growth promoting activity of Serratia nematodiphila N11-0928 on black pepper (Piper nigrum L.).World J.Microbiol.Biotechnol.**27**:259-265.*
32. *Virdi J S, Dwivedi S and Gupta V. 1994. Antifungal activity and nature of antifungal antibiotic produced by Bacillus subtilis. Micron 94, 35th AMI Conference held at DFRL, Mysore.*
33. *Quesado C A, Vtzinger D and Jimenez F. 1996. Antimicrobial activity of Bacillus USR 236 and its potential use as biological control. Aronivas-de Biologea-e-Technologia **39**(3): 613-621.*
34. *Khan M R and Khan S M. 2002. Effects of root dip treatment with certain phosphate solubilizing microorganisms on the fusarial wilt of tomato. Bioresource Technology **85**: 213-215.*
35. *Ramirej A R, Abarca E, Aquilar U G, Hayward- Jones P M and Barboza J E. 2004. Antifungal activity of Bacillus thuringiensis chitinase and its potential for the biocontrol of phytopathogenic fungi in soyabean seeds. Journal of Food Science **69**(5): M131-M134.*
36. *Calvo P, Orrillo E O, Romero E M and Zuniga D.2010.Characterization of Bacillus isolates of potato rhizosphere from Andean soils of Peru and their potential PGPR characteristics.Brazilian Journal of Mocrobiology **41**:899-906.*
37. *Cazorla F M, Romero D, Garcia A Perez, Lugtenberg B J J, Vincente A and Bloemberg G. 2007. Isolation and characterization of antagonistic B. subtilis strains from avocado rhizoplane displaying biocontrol activity. Journal of Applied Microbiology 1-10.*
38. *Idris H A, Labuschagne N and Korsten L.2007.Screening rhizobacteria for biological control of Fusarium root and crown rot of sorghum in Ethiopia.Biol.Control **40**(1):97-106.*

APPENDICES

Table 1: Colony Morphology and Gram's Reaction of the Selected Phosphate Solubilizing Bacteria Isolated from Picrorhiza Kurroa

Isolates	Colony Morphology	Gram's Reaction	Cell Shape
Pk3A	Irregular, flat, lobate, creamish	+	rods
Pk4a	Irregular, flat, lobate, creamish	+	rods
PkH(4)	Irregular, flat, lobate, creamish	+	rods
PkH(5)	Irregular, flat, lobate, whitish	+	rods
Pk6(B)	Irregular, raised, lobate, creamish	+	rods
Pk6(c)	Circular, convex, entire, creamish	+	rods
Pk7(B)	Irregular, flat, lobate, creamish	+	rods
Pk8(A)	Circular, flat, entire, creamish	+	rods
Pk8(e)	circular, convex, entire, yellowish	+	rods
Pk9(B)	Circular, convex, entire, yellowish	+	rods

Table 1: Contd.,			
Pk11(c)	Irregular, flat, undulate whitish	+	rods
Pk12(a)	Irregular, umbonate, curled, creamish	+	rods
Pk12(b)	Circular, convex, entire, creamish	+	rods
Pk12(c)	Irregular, raised, lobate, creamish	+	rods
Pk12(d)	Circular, convex, entire, creamish	+	rods
Pk13(a)	Irregular, flat, lobate, creamish	+	rods
Pk13(b)	Irregular, flat, lobate, creamish	+	rods
Pk13(c)	Circular, convex, entire, creamish	+	rods
Pk14(a)	Circular, convex, entire, creamish	+	rods
Pk14(b)	Circular, convex, entire, creamish	+	rods
Pk14(c)	Circular, convex, entire, creamish	+	rods
PkR(2)*	Circular, convex, entire, yellowish	+	rods
PkR(21)*	Irregular, raised, lobate, creamish	+	rods
Pk(22)*	Irregular, raised, lobate, whitish	+	rods
PkR(32)*	Circular, raised, entire, creamish	+	rods
PkR(33)*	Circular, convex, entire, whitish	+	rods
PkR(34)*	Circular, convex, entire, yellowish	+	rods
PkR(5a)*	Circular, raised, entire, creamish	+	rods
PkR(5e)*	Irregular, umbonate, curled, creamish	+	rods
PkR(6a)*	Irregular, raised ,undulate, creamish	+	rods
PkR(7a)*	Irregular, flat, lobate, creamish	+	rods
PkR(7b)*	Irregular, flat, undulate, whitish	+	rods
PkR(7c)*	Irregular, flat, undulate, whitish	+	rods
PC2	Irregular, flat, undulate, yellowish	+	rods
PC3	Irregular, flat, undulate, whitish	+	rods
PC4	Irregular, raised, erose, whitish	+	rods
PC7	Irregular, flat, erose, yellowish	+	rods
PC8	Irregular, flat, undulate, yellowish	+	rods
PC9	Irregular, flat, erose, yellowish	+	rods
PC13	Irregular, flat, erose, yellowish	+	rods

***:** Endorhizobacteria

Table 2: Screening of the Phosphate Solubilizing Bacterial Isolates for Multifarious Plant Growth Promoting Activities

Isolates	Phosphate Solubilization		Siderophore Production		Indole -3- Acetic Acid (µg/ml) Production
	Phosphate solubilization index (PSI) **	Quatitative P-Estimation (mg/l)●	Qualitative Siderophore Estimation (Zone Size, mm)	Quantitative estimation (%Siderophore Unit)	
Pk3A	2.45	105.00	12.30	16.66(4.08)	13.00
Pk4a	2.27	135.00	7.30	14.96(3.86)	19.00
PkH(4)	2.56	115.00	13.00	15.57(3.94)	21.00
PkH(5)	2.61	130.00	5.00	12.05(3.46)	5.00
Pk6(B)	2.88	120.00	6.00	9.70(3.10)	14.00
Pk6(c)	2.50	100.00	8.00	14.28(3.77)	6.00
Pk7(B)	2.58	120.00	20.00	21.76(4.66)	14.00
Pk8(A)	2.35	135.00	4.00	4.08(2.00)	12.00
Pk8(e)	2.43	95.00	3.00	0.75(0.866)	3.00
Pk9(B)	2.50	95.00	5.00	21.30 (4.61)	6.00
Pk11(c)	3.00	125.00	17.60	16.41(4.04)	21.00
Pk12(a)	2.50	75.00	14.60	21.30(4.61)	10.00
Pk12(b)	2.80	50.00	10.00	27.21(5.21)	14.00
Pk12(c)	2.43	85.00	20.00	25.07(5.00)	13.00
Pk12(d)	2.38	100.00	19.00	16.16(4.01)	22.00
Pk13(a)	3.16	95.00	17.30	17.45(4.17)	6.00

Table 2: Contd.,					
Pk13(b)	2.80	80.00	11.00	10.42(3.21)	11.00
Pk13(c)	2.56	120.00	4.00	8.20(2.86)	3.00
Pk14(a)	3.00	90.00	8.00	15.38(3.92)	16.00
Pk14(b)	4.05	205.00	11.00	8.20(2.86)	21.00
Pk14(c)	3.42	40.00	5.00	10.42(3.22)	4.00
PkR(2)*	2.36	80.00	12.00	16.09(4.01)	9.00
PkR(21)*	2.40	100.00	8.30	14.61(3.820	5.00
PkR(22)*	2.32	55.00	7.60	13.61 (3.68)	8.00
PkR(32)*	2.54	110.00	19.60	17.31(4.15)	0.00
PkR(33)*	2.33	55.00	24.00	16.54(4.06)	16.00
PkR(34)*	2.35	70.00	14.30	8.64(2.93)	30.00
PkR(5a)*	2.78	90.00	11.30	12.38(3.50)	4.00
PkR(5e)*	2.62	90.00	11.60	16.00(3.99)	4.00
PkR(6a)*	2.65	100.00	19.60	17.03(4.12)	22.00
PkR(7a)*	2.73	320.00	12.00	0.75(0.86)	21.00
PkR(7b)*	2.82	20.00	5.00	2.04(1.42)	30.00
PkR(7c)*	3.30	90.00	7.00	11.31(3.35)	11.00
PC2	3.00	80.00	16.60	16.16(4.01)	0.00
PC3	2.75	90.00	10.30	6.00(2.42)	21.00
PC4	2.60	180.00	11.00	9.70(3.11)	18.00
PC7	3.07	75.00	21.00	18.64(4.30)	24.00
PC8	2.66	80.00	18.00	16.09(4.00)	11.00
PC9	3.05	50.00	21.00	17.70(4.20)	28.00
PC13	3.42	60.00	18.30	12.03(3.46)	16.00
$CD_{0.05}$	1.05	4.05	3.08	0.36	2.55
(r= 0.09)		(r= 0.10)			

* **:** Endorhizobacteria

**: $\frac{Clear\ zone\ diameter(colony+holozone)}{Growth\ diameter}$;

T-C; Where, T= Inoculated PVK with TCP, C (uninoculated PVK with TCP)

% Siderophore unit= $\frac{Ar-As}{As}$X 100

A_r = Absorbance at 630nm of reference; A_s = Absorbance at 630 nm of test sample;

Table 3: Screening of the Isolates for Chitinase Activity and HCN Production

Isolates	Chitinase Activity**	HCN*** Production
Pk3A	++	++
Pk4a	+++	+
PkH(4)	++	+
PkH(5)	++	+
Pk6(B)	++	-
Pk6(c)	+	-
Pk7(B)	++	+
Pk8(A)	+	-
Pk8(e)	-	-
Pk9(B)	-	+
Pk11(c)	++	++
Pk12(a)	++	++
Pk12(b)	+	+
Pk12(c)	++	+
Pk12(d)	++	+

Table 3: Contd.,		
Pk13(a)	+	+
Pk13(b)	+	-
Pk13(c)	+	-
Pk14(a)	-	+++
Pk14(b)	-	++
Pk14 (c)	+	+++
PkR (2)*	+	++
PkR (21)*	-	+
PkR (22)*	++	-
PkR (32)*	++	-
PkR (33)*	+	+
PkR (34)*	++	+
PkR (5a)*	++	-
PkR (5e)*	++	+
PkR (6a)*	+++	+
PkR (7a)*	+++	-
PkR (7b)*	+++	+
PkR (7c)*	++	+
PC2	++	+
PC3	-	-
PC4	-	++
PC7	++	+++
PC8	++	++
PC9	-	-
PC13	+	-

***:** Endorhizobacteria

**-: no zone +:zone size 0-15mm, ++:zone size 15-30mm and +++:zone size 30-45mm

*** - : No colour change of the filter paper from yellow to brown; + : change in colour on the edge of the filter paper; ++ : change in colour only half of the filter paper; +++ : change in colour of the complete filter paper from yellow to orange brown

Table 4: Screening of Phosphate Solubilizing Bacterial Isolates for Antifungal Activity Against Different Fungal Pathogens

Isolates	**Antifungal Activity of the Representative Bacterial Isolates				
	Fusarium Oxysporum	Alternaria Solani	Pythium Aphanidermatum	Sclerotium Rolfsii	Dematophora Necatrix
Pk3A	-	-	-	+++	+
Pk4a	+	++	-	-	+++
PkH(4)	+	+++	++	-	+++
PkH(5)	+	++	+	+	+++
Pk6(B)	+	++	-	-	+++
Pk6(c)	-	-	-	-	-
Pk7(B)	+	++	+	-	+++
Pk8(A)	-	-	-	-	-
Pk8(e)	-	-	-	-	-
Pk9(B)	-	-	-	-	++
Pk11(c)	+	+++	-	-	+++
Pk12(a)	+	-	-	-	++
Pk12(b)	+	-	-	-	+
Pk12(c)	-	-	-	-	-
Pk12(d)	+	++	+	+	+++

Table 4: Contd.,					
Pk13(a)	+	++	+	+	+++
Pk13(b)	+	+	+	+	+++
Pk13(c)	+	-	-	-	-
Pk14(a)	-	-	-	++	++
Pk14(b)	-	-	-	-	-
Pk14(c)	-	-	+	+++	-
PkR(2)*	-	-	-	-	-
PkR(21)*	+	-	+	+++	-
PkR(22)*	-	-	+	-	+++
PkR(32)*	-	-	-	-	-
PkR(33)*	+	++	++	+	+++
PkR(34)*	+	+++	+	+++	-
PkR(5a)*	-	-	-	-	-
PkR(5e)*	-	++	+	-	-
PkR(6a)*	-	-	-	-	-
PkR(7a)*	+	-	+	+	+
PkR(7b)*	-	-	-	-	-
PkR(7c)*	-	-	-	-	-
PC2	+	-	-	+++	-
PC3	+	+++	++	+	-
PC4	+	+++	+	++	+++
PC7	+	-	-	-	+++
PC8	+	+++	++	+	++
PC9	+	-	-	-	++
PC13	+	++	-	+	+

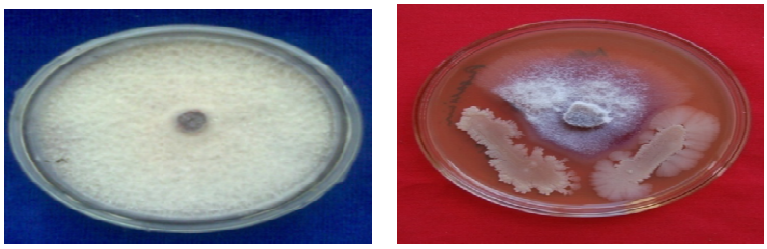
* Endorhizobacteria

** $I = \frac{C-T}{C} \times 100$

Where, I = Per cent growth inhibition, C = Growth of fungus in control,T = Growth of fungus in treatment - : No inhibition; + : contact growth inhibition; ++ : 45 to 65 per cent growth inhibition; +++ : 65 to 85 per cent growth inhibition

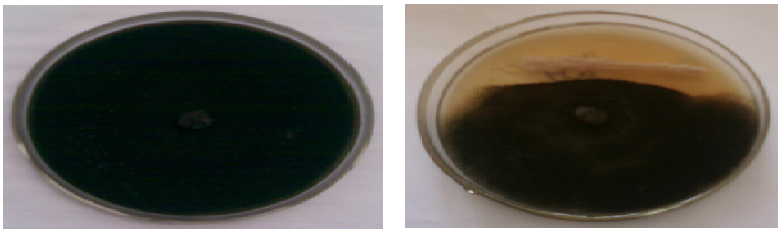
Dematophora Necatrix (Control) **Dematophora Necatrix + Bacterial**

Plate 1: Antifungal Activity Against Dematophora Necatrix



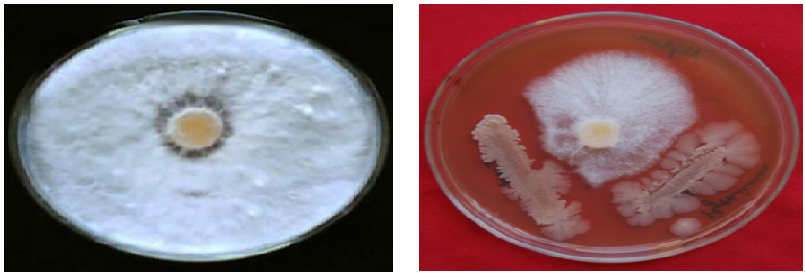
Fusarium Oxysporum* (Control)** ***Fusarium Oxysporum **+ Bacterial Isolates**

Plate 2: Antifungal Activity Against *F.* Oxysporum



Alternaria Solani (Control) **Alternaria Solani + Bacterial Isolate**

Plate 3: Antifungal Activity against Alternaria Solani



Pythium Aphanidermatum (Control) **Pythium Aphanidermatum + Bacterial**

Plate 4: Antifungal Activity against *P. Aphanidermatum*